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Giant Viruses

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Giant Viruses

The recent discovery of really, really big viruses is changing views about the nature of viruses and the history of life

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The common view of viruses, mostly true, is of tiny burglars that sneak into cells, grab the biosynthetic controls and compel the cell to make huge numbers of progeny that break out of the cell and keep the replication cycle going. Viruses are supposed to be diminutive even compared to cells that are just a micrometer (1,000 nanometers) in diameter. They are supposed to travel light, making do with just a few well-adapted genes.

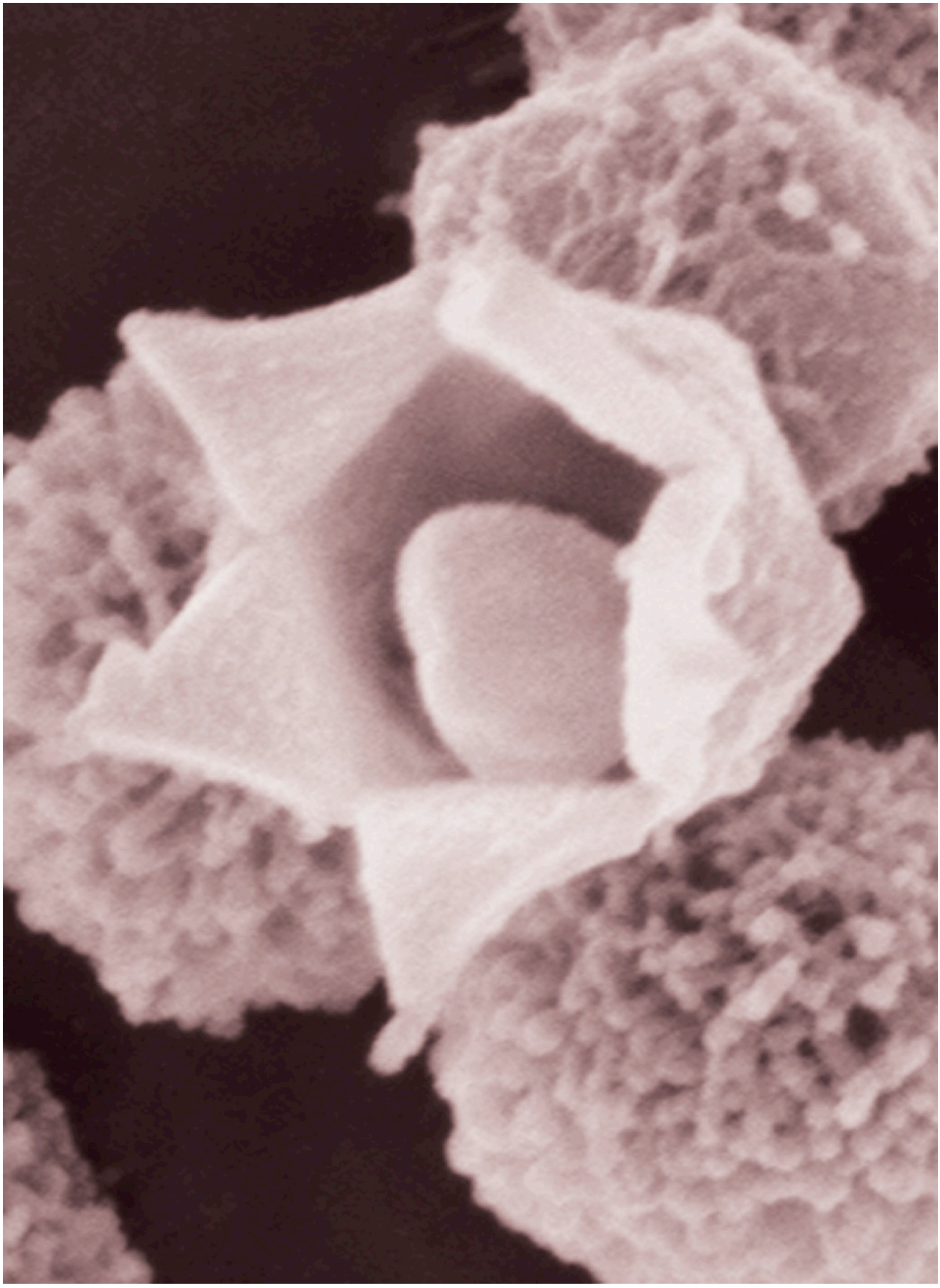
In 1992, a new microorganism was isolated from a power-plant cooling tower in Bradford, England, where Timothy Rowbotham, a microbiologist at Leeds Public Health Laboratory, was seeking the causative agent of a local pneumonia outbreak. His search led to the warm waters of the cooling tower, a known reservoir for bacterial pathogens in the *Legionella* genus, which are the cause of the pneumonialike Legionnaire's disease. Particles present in the sample were mistakenly identified as bacteria. Gram positive and visible under the microscope as pathogens within the particle-gobbling amoeba *Acanthamoeba polyphaga,* the entities surprisingly did not generate any product from the gene-amplifying polymerase chain reaction technique using universal bacterial primers.

Eleven years later, in 2003, the mystery organism received a new identity and a new name, *Acanthamoeba polyphaga* Mimivirus, for microbe-mimicking virus. Mimivirus is the largest virus ever discovered. Giant viruses had been known for a few years, many of them in a group termed nucleo-cytoplasmic large DNA viruses (NCLDVs). This group features several other virus families, including *Poxviridae,* which infects vertebrates (for example, smallpox virus) and invertebrates, the aquatic viruses *Iridoviridae* and *Phycodnaviridae,* and the vertebrate virus *Asfarviridae.* Giant viruses are considered to be ones with genomes larger than 300 kilobase pairs and with capsid diameters of about 200 nanometers or more.

Mimivirus is a giant among giant viruses, with a diameter of 750 nanometers. It possesses a genome, truly outsized by viral standards, of 1.2 million base pairs, coding an outlandish 1,018 genes. For comparison, the smallest free-living bacterium, *Mycoplasma genitalium,* is just 450 nanometers in diameter and possesses a genome half the size of that in mimivirus, while coding just 482 proteins. The record tiniest cellular organism, *Hodgkinia cicadicola,* a parasite in cicadas that was described in 2009, has a genome of just 140,000 base pairs, coding a paltry 169 proteins. *H. cicadicola* is unable to live on its own, being entirely dependent on the lush environment of specialized cicada cells. Viruses are generally not considered living organisms (although for a consideration of their position in the phylogenetic tree of life, see the sidebar box in the section headed "Origins"), yet mimivirus brings a bigger blueprint and more lumber to the replication process than the living *H. cicadicola* and many other bacteria.

Most giant viruses have only been discovered and characterized in the past few years. There are several reasons why these striking biological entities remained undetected for so long. Among the most consequential is that the classic tool for isolating virus particles is filtration through filters with pores of 200 nanometers. With viruses all but defined as replicating particles that occur in the filtrate of this treatment, giant viruses were undetected over generations of virology research. (Mimivirus disrupted this evasion tactic by being so large it was visible under a light microscope.) Standard plaquing procedures failed to report the presence of giant viruses because the large particles bogged down in the soft agar of the plaquing medium, disrupting diffusion and the formation of visible plaques. An additional explanation for the elusiveness of the largest viruses is that many infect protists, which have received far less research attention than plants and animals.

Figure 1. Giant viruses, which some call giruses in acknowledgment of their many unique features, comprise an ancient line that has been hidden in plain sight. Ironically, their gigantic size kept the giant viruses from being isolated by the usual filtration techniques. Shown at right is the biggest of them all (so far), mimivirus. The giant viruses possess exotic genes, lifestyles and physiological features, such as the stargate portal of mimivirus, shown here wide open after the release of the virus's genetic cargo. Researchers probing the huge genomes of the giant viruses with bioinformatics tools are learning that the picture of early life may need adjustment to take account of these fascinating biological entities.
Image courtesy of Abraham Minsky, Weizmann Institute of Science.



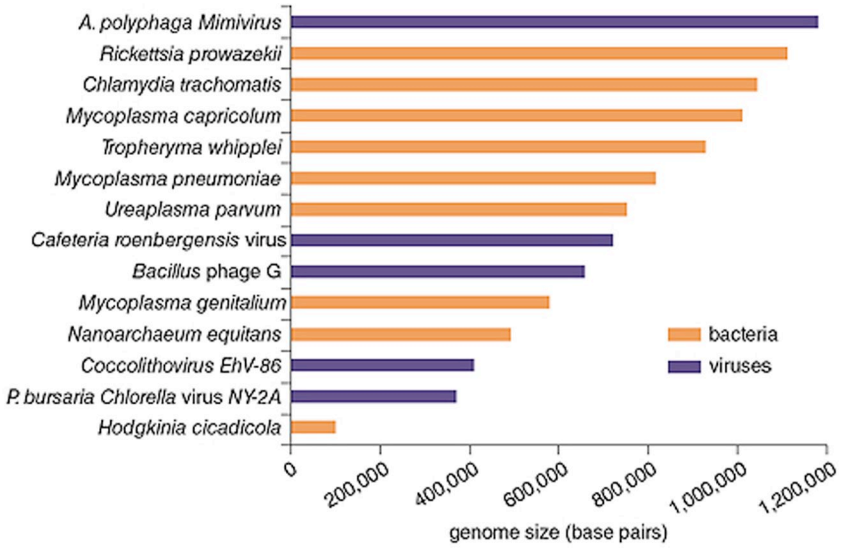


Figure 2. The genomes of giant viruses can be larger than those of some bacteria. These genomes are also packed with novelty. In the case of mimivirus *(top)*, 86 percent of its predicted protein-coding sequences have no known homologs with other genes in gene databases. Illustration by Barbara Aulicino. Includes data provided by Alan Cann.

With the spotlight finally on them, the giant viruses are delivering striking lessons in viral physiology and ecology, not to mention challenging long-held assumptions about the shape of the phylogenetic tree of life.

Big Family

Central to the placement of giant viruses in the tree of life is the presence of numerous previously unknown virus-encoded gene families. A recent reconstruction of NCLDV evolution suggests a common ancestor that likely contained a minimum set of 47 genes that have left traces in modern viral genomes. The NCLDVs then evolved by losing some genes, duplicating others and acquiring genes from their hosts and other organisms. The giant viruses, like other viruses, are genetic pickpockets, grabbing genes from their hosts over eons. Interpretation of viral phylogenetic reconstructions is therefore rife with puzzles. Yet a faded outline of evolutionary history is visible. Analysis of 45 giant viruses identified five genes common to all of the NCLDV viruses and 177 additional genes that are shared by at least two of the virus families. The ancient genetic signal, however, is very weak. Consider that in a selection of three viruses in the *Phycodnaviridae* family, 14 genes in common indicate a shared evolutionary history, yet within the sprawling genomes of these three biological entities, more than *1,000* total genes exist.

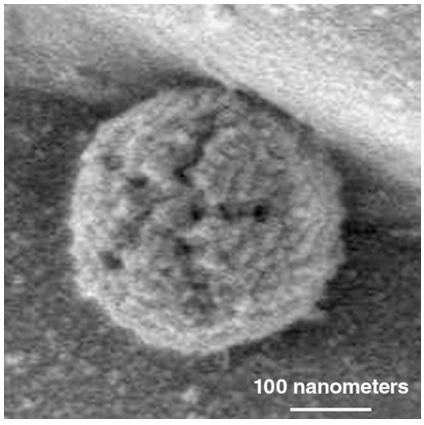


Figure 3. Mimivirus is covered with fibers, visible above as a woolly surface and shown in more detail in the 3D model on the cover of this magazine. The stargate substructure lacks fibers and is visible as an indentation in the fiber coat.
Image courtesy of Abraham Minsky, Weizmann Institute of Science.

Mimivirus is an appropriate representative of the giant viruses, exhibiting a variety of unique properties that point to the diversity of the known giant viruses and those soon to be discovered. The mimivirus virion particle (the complete assembly of genetic material and protein coat) has an icosahedral core of ~500 nanometers covered with a ~140-nanometer layer of closely packed fibers. The fibers have not been completely characterized but based on the presence of collagen-like genes in the mimivirus genome, they may be a form of substituted collagen, the fibrous constituent of animal connective tissue. Mimivirus is the only NCLDV member known to have this peripheral fiber layer. Another singular feature of the mimivirus virion is a prominent five-fold star-shaped structure on one icosahedral vertex called the stargate portal.

Research suggests that mimivirus ingested by an amoeba enters the cell in a membranous compartment that fuses with lysosomes, which are digestive organelles. The activity of lysosomal enzymes is predicted to cause the stargate portal to open. An internal membrane within the mimivirus then apparently fuses with the surrounding membrane compartment, forming a conduit through which the viral genome passes into the cytoplasm of the host. A viral-assembly complex called a replication factory then forms in the cytoplasm around the viral core. The replication factory expands until it occupies a large fraction of the cell volume six hours after the initial infection.

In the replication factory, empty, partially assembled viral capsids without fibers undergo DNA packaging. Curiously, DNA packaging is reported to occur through faces of the viral capsid that are not the stargate—DNA entry into and exit from the virion apparently occur at different portals, which is very unusual for a virus.

In 2008, a new strain of mimivirus was isolated from another cooling tower, this one in Paris. With a slightly larger genome than mimivirus, the new strain was named mamavirus, and it brought with it a surprise—a parasite virus named Sputnik. Viral satellites, which are quite common, are subviral agents consisting of small amounts of nucleic acid whose replication depends on a viral genome. In this case, the viral companion may be imperfectly named, since Sputnik appears to be not merely a satellite but a legitimate parasite of its host. When present, it interferes with the infectivity of mamavirus in amoebae and appears to cause the formation of defective mamavirus virions, which is not the case for traditional satellite viruses. This unprecedented property and other features of its lifestyle have led to the proposal of a new group and new name, *virophage,* for viruses that parasitize giant viruses. A paper published in April 2011 reports on a new strain of mimivirus infected by a new strain of virophage. In the busy enterprise of giant virus research, news comes fast and often.

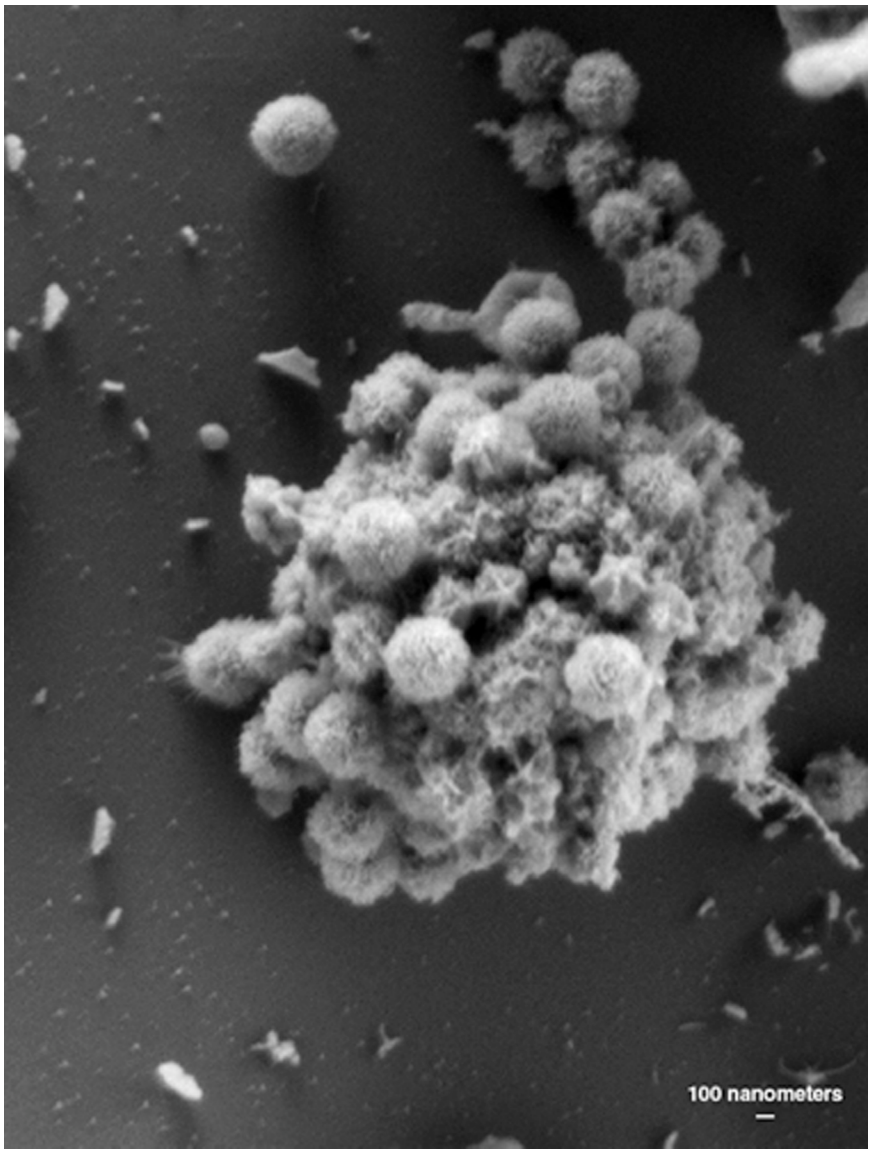


Figure 4. Like many viruses, mimivirus generates progeny in a complex called a replication factory. Both coated and uncoated virus particles are visible in this electron micrograph of a virus factory isolated from an amoeba cell 8 hours after infection. Image courtesy of Abraham Minsky, Weizmann Institute of Science.

Origins

The origin of the NCLDV group is controversial. Upon discovery of mimivirus, some researchers, addressing its huge number of genes with no detectable resemblance to any cellular genes—86 percent of the total coding sequences in mimivirus—concluded that NCLDVs should be considered a fourth domain of life alongside the Archaea, Bacteria and Eukarya. It has been suggested that some NCLDV genes may have arisen from the same ancient gene pool from which sprang the prokaryotes and eukaryotes.

An interesting pair of contrasting hypotheses suggest that given the size and complexity of NCLDV genomes, either the ancestor of modern NCLDVs may have given rise to the eukaryotic genome itself, or decay of a eukaryotic genome may have produced the genome of the NCLDV family. Horizontal gene transfer between virus and host has also played an important role in the evolution of NCLDVs (and their host cells), beginning far back in biological history.

Given their distinctness in morphology, ecology, genome size and gene uniqueness, a new name has been proposed for the giant viruses—*giruses.* The semantic and scientific goal of the new name is to emphasize the unique properties of large DNA viruses, which likely represent a unique and shared evolutionary history. The term *virus (poison* to the Greeks) is more than a hundred years old. In the time since the word was coined, a huge diversity of viral agents have been discovered with highly divergent lifestyles, physiologies and replication strategies. A collective group name worked satisfactorily when viruses were definable as small, relatively simple genetic agents dependent on hosts for their replication. The term seems less adaptable as the family of large DNA viruses is characterized in increasing detail, and evolutionary relationships between the members become increasingly visible based on deep bioinformatic analysis of their very large, complex genomes. In the past couple of years, the term *girus* has seen increasingly regular appearances in the virological literature.

Ecological Role

Part of the characterization of novel biological actors is consideration of their role in shaping their environment. Giant viruses were not overlooked because they are rare, nor are they marginal players in their ecological spheres. In recent years, the emergent field of metagenomics has opened a new window on understanding ecosystems. The metagenome of an environment is the sum of all genomes of the organisms present. Using a technique called shotgun sampling, environmental material is collected, the DNA in the unsorted sample is randomly sheared, and the resulting fragments are sequenced. Overlapping sequences are then aligned to reconstruct genes. Some of the resultant genes can be identified by reference to gene databases, many cannot. The very high number of unidentified genes that are found in metagenomic studies is a driver of the surging biodiversity movement. Through metagenomics, we are in the peculiar position of knowing that the number of species we *don't* know about is vast.

In a striking demonstration of the power of environmental sequencing, Mya Breitbart, Forest Rohwer and colleagues demonstrated in 2002 that 200 liters of seawater contains more than 5,000 different viruses, essentially all of them unknown species. In another study, the Global Ocean Sampling Expedition sampled waters from Nova Scotia

Where do viruses belong in the phylogenetic tree of life—or do they belong at all?

A play-by-play

In a 2006 article in the journal *Genome Biology,* Jean-Michel Claverie of the Structural and Genomic Information Laboratory in Marseille, France, made a provocative contribution to a long-running debate about whether viruses are "life." "I believe that the virus factory should be considered the actual virus organism when referring to a virus. Incidentally, in this interpretation, the living nature of viruses is undisputable, on the same footing as intracellular bacterial parasites." The virion particle would then be just a reproductive form, a stage in the "life" of a virus before it clothes itself in the metabolic apparatus of a host cell, directs the construction of the internal virus factory and takes up the business of reproducing itself like any other form of unicellular life.

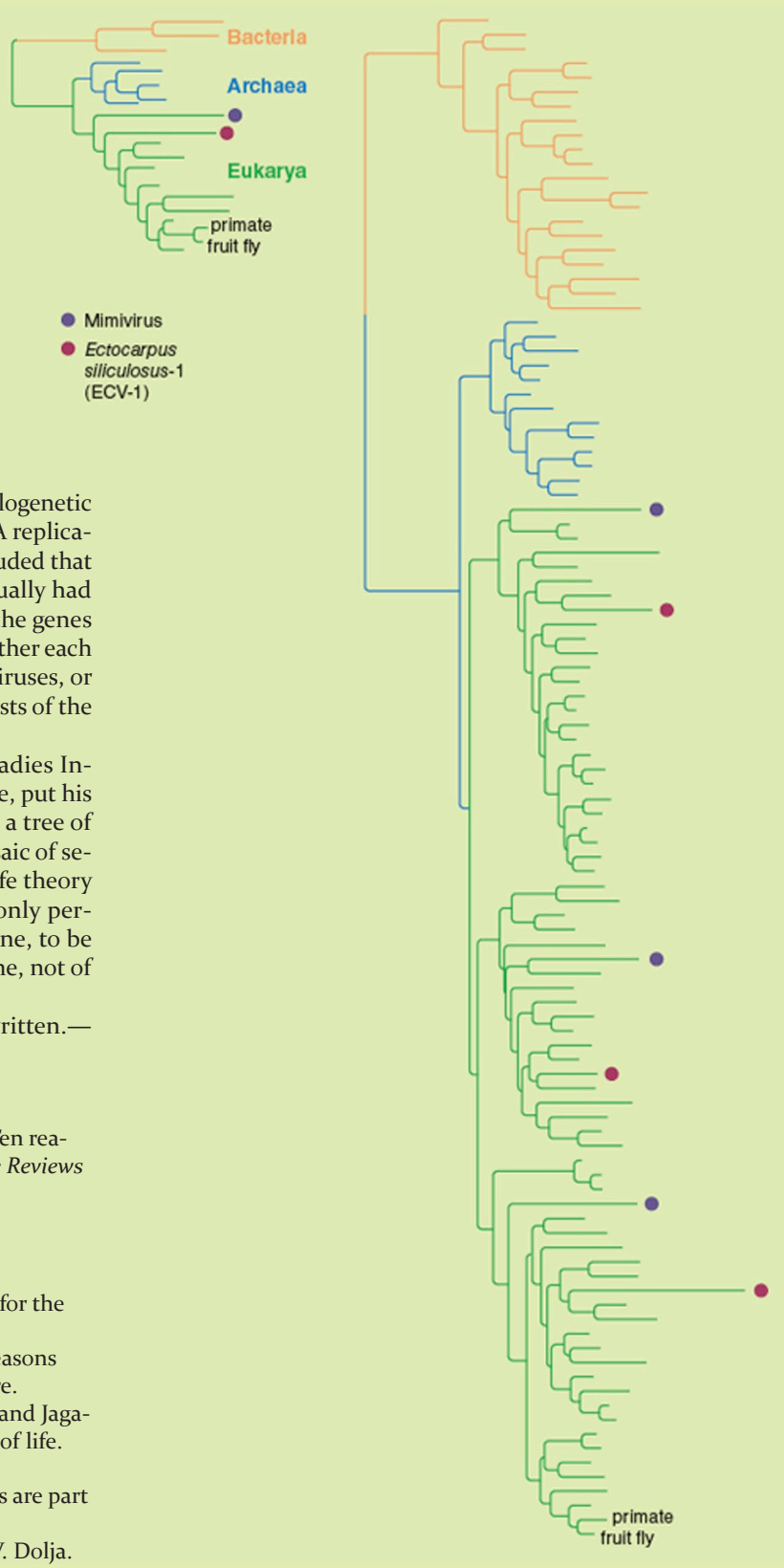
In 2009, David Moreira and Purificación López-García at the Unité d'Ecologie Systématique et Evolution recalled this argument in a rousing bout of biological reasoning (one responder called it "courageous," slyly not letting on whether tongue was in cheek) published in *Nature Reviews Microbiology.* In "Ten reasons to exclude viruses from the tree of life," the authors argued that not only were these genetic entities not alive, they also had no place in any phylogenetic tree linking extant organisms to the common ancestor of all life. Among the factors that stimulated the writing of their article was the discovery of the giant mimivirus in 2003, and the idea put forward by some analysts of the huge mimivirus genome that giant viruses might actually represent a fourth branch in the tree of life alongside the three domains of Archaea, Bacteria and Eukarya. Moreira and López-García were having none of it. Their 10 reasons for excluding viruses from the tree of life ranged from blunt to subtle. Viruses are not alive. Their genes are stolen. There is no single gene shared by all viruses—no ancestral viral lineage. Perhaps most pointedly, viruses are polyphyletic: A phylogenetic tree is a conceptual representation of evolutionary relationships that can only be inferred by studying inherited characteristics from an unbroken line back to the common ancestor of the taxa. Viruses, instead, originate here and there in the evolutionary tree and go on to pick up genetic cargo via horizontal gene transfer from hosts.

In a subsequent issue of the journal, 10 pages were devoted to a spirited correspondence adjudicating the position of Moreira and López-García and the old question: How to think about viruses? It is impossible to do justice here to all of the arguments made, but an overview may give a sense of the flavor and interest of the debate, as well as showing how the discussion is being remodeled by new findings about giant viruses. Citations for the entire exchange are given on the next page.

Acting as a sort of moderator, Jesús Navas-Castillo opened the tourney with an appeal to clearheadedness. "Linking vague philosophical definitions of 'life' to inclusion in the tree of life seems dubious at best." Giving some of the original arguments short shrift, he then took on a factual premise. To the assertion that high rates of horizontal gene transfer and high recombination rates in viruses mean that "a set of genes that is found together in a viral genome at a given time has little chance to remain linked after a small number of generations," Navas-Castillo countered strongly: "Comparative genomics does not support such volatility. The well-defined virus-specific gene ensembles hold together for eons, as has been shown for the [giant viruses] and picorna-like RNA viruses."

In the next volley, among other points, Jean-Michel Claverie and Hiroyuki Ogata took on the rejection of viruses because of their polyphyletic origin. They noted that the discussion was prompted initially not by the nature of viruses in general, which are massively polyphyletic, but by the characteristics of the newly discovered giant viruses. In fact, they pointed out, they had proposed the term "girus" to recognize that the properties and perhaps evolutionary origins of large DNA viruses were so distinctive that it was unreasonable to lump them indiscriminately with all other viruses. "Asking if ancestral giruses might not be part of the underground reticulated roots of a 'forest of life' is a legitimate question." They went on to observe that denigrating viruses as robbers of host genes is not pertinent when 86 percent of mimivirus genes are "genomic dark matter" that does not resemble any known cellular genes. Furthermore, they presented a phylogenetic tree of a DNA replication protein in which mimivirus and another giant virus, Ectyocarpus siliculosus virus-1 (ESV-1) evidently branched near where life first separated into three domains.

In a response to that phylogenetic tree, the original authors parried with a richer tree of more species—106 taxa versus 20—and including a generous selection of target gene homologs from mimivirus and ESV-1 hosts, which would be likely sources



A phylogenetic tree of 20 taxa depicts the place of a DNA replication gene in mimivirus and the giant virus ECV-1 *(left).* Their branches arise in deep evolutionary time, during the era when Eukarya and Archaea branched into separate domains. A more complex tree of 106 taxa *(right),* augmented with additional homologs of the DNA replication protein, tells a somewhat different story, with the gene homologs evidently most closely related to homologous genes in organisms from much later branches of widely divergent trunks in the phylogenetic tree. Illustration by Barbara Aulicino. Data adapted from Claverie and Ogata correspondence and from López-García and Moreira response, cited below.

of horizontal gene transfer. They also showed the phylogenetic position of not one but three copies of the same DNA replication gene found in the viruses (*see figure)*. They concluded that the gene group was not merely polyphyletic but actually had roots in distantly related eukaryotic groups before the genes were burgled by the viruses. (Not entirely clear is whether each of the groups contributed a copy of the gene to the viruses, or whether viral infection left evidence behind in the hosts of the separate groups.)

Didier Raoult at the Unité de Recherche en Maladies Infectieuses et Tropicales Émergentes, Marseille, France, put his foot down with a decisive "there is no such thing as a tree of life." Current organisms are chimeric, "made of a mosaic of sequences of different origins that makes the tree of life theory obsolete." The rooted tree imagined by Darwin is only pertinent in the genomic age if constructed gene by gene, to be used for deducing the evolutionary history of the gene, not of the life form.

The last word on this debate? That is yet to be written.—*The Editors*

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Correspondence:

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Ludmir, Ethan B., annd Lynn W. Enquist. Viral genomes are part of the phylogenetic tree of life.
Koonin, Eugene V., Tatiana G. Senkevich and Valerian V. Dolja. Compelling reasons why viruses are relevant for the origin of cells.
Response: López-García, Purificación, and David Moreira. Yet viruses cannot be included in the tree of life.

Figure 5. Viruses make important contributions to the ecosystems in which they are found. The giant viruses are no exception. *Cafeteria roenbergensis* virus, or CroV, infects microzooplankton that feeds on phytoplankton. The phytoplankton *Emiliani Huxleyi* forms giant blooms in oceans worldwide. Termination of the blooms by infection with the giant *E. huxleyi* virus, or EhV, results in deposition of the calcium-carbonate skeletons of the phytoplankton, leading to formations like the White Cliffs of Dover. Chemical products from the decay of *E. huxleyi* reach the atmosphere, where they seed rain clouds. Loop Images/Corbis

to the eastern Pacific during a two-year circumnavigation employing a more targeted approach of using known sequences of protein products, such as specific DNA polymerase fragments, to query metagenomic DNA and thus to determine the prevalence of species. In 86 percent of sample sites, mimivirus relatives were the most abundant entities after bacteriophage. Thus giruses are common in nature and it is clear that there are many more awaiting discovery.

Their role in shaping their environment is also becoming clear. More than half of all photosynthesis on Earth is carried out by photosynthetic microorganisms, including cyanobacteria and microalgae, which are collectively referred to as phytoplankton. It is estimated that at any one time, 20 percent of all phytoplankton cells are infected by viruses, including giant viruses in numbers that are unknown but clearly of quantitative importance. Central to the ecology of ocean systems, and essential to the well-being of the Earth, are microzooplankton that feed on phytoplankton and are known as protistan grazers. To date just one virus has been shown to infect a protistan grazer, *Cafeteria roenbergensis* virus, or CroV—a giant virus (730 kilobase pairs, 544 predicted protein-coding genes). Interestingly, CroV also has a virophage associated with it.

Phytoplankton are intimately linked to another giant virus, with consequences for sea, terrain and sky as well as phytoplankton community ecology. *Emiliani huxleyi* is one of the most abundant unicellular photosynthesizing algae in the oceans. Cells of *E. huxleyi* produce tiny scales of calcium carbonate, which, given the abundance of these microalgae, plays an important role in the carbon cycle of the ocean. *E. huxleyi* periodically forms huge blooms as large as 100,000 square kilometers in both the northern and southern hemispheres. A giant virus that infects *E. huxleyi,* called EhV (407 kilobase pairs, 472 predicted coding sequences), is largely responsible for terminating these blooms. The demise of *E. huxleyi* blooms releases massive amounts of organic matter, including detached calcium carbonate scales, which form large deposits. A striking example is the White Cliffs of Dover.

The termination of *E. huxleyi* blooms also results in the release of a chemical that is altered by other microorganisms, producing vast amounts of dimethylsulfide, which accounts for the smell we associate with sea water. When dimethylsulfide reaches the atmosphere, it induces cloud formation and rain. Thus, EhV infection of its host plays a role in the ecology, geology and climate of its environment.

Giant Human Virus?

The mimivirus particles in samples from the Bradford cooling tower were discovered among bacteria with the potential to cause pneumonia, and consequently there has been interest in the question of whether mimivirus might be a human pathogen.

At first glance, the odds seem unlikely that a pathogen of amoebae could make the leap to human infection. Humans and amoebae are separated by 800 million years of evolution, and an infective leap across a chasm that large would be highly unusual in virology. Typically, viral infections are highly specific for their hosts. This specificity is a result of the requirement that the virus co-opt the synthetic machinery of the host cell in order to replicate. Doing so requires many intricate and specific macromolecular interactions between viral and host components at every stage of infection, from cell entry to virus replication, which requires hijacking most of the cell's biochemical and molecular machinery, often coupled with additional inhibition of cell defenses. It is not surprising, then, that quite similar viruses, such as HIV, the cause of AIDS, and the simian strain, SIV, do not cross-infect their closely related primate targets.

Yet mimivirus often challenges the usual rules. It gains entry into phagocytic cells (such as amoebae and possibly human macrophages) when the scavengers engulf it. It exits the vacuole that surrounds it after engulfment by relatively nonspecific membrane fusion. From that point, its huge complement of more than 1,000 genes may confer the ability to hijack or replicate cell functions beyond the ability of viruses of lesser genetic endowment.

To date, there is only slim evidence that mimivirus may infect humans. Studies in one Canadian laboratory hint that the question should remain open. Other studies find no evidence for human infection. A review in 2009

proposed that the prudent course is to treat mimivirus provisionally as a biosafety class 2 pathogen, the designation for pathogens that cause only mild disease or that are unlikely to be communicated as aerosols in a lab.

The giant NCLDV viruses probably have an ancient evolutionary history, but they are among the newest things on the scene for virologists. It should be noted that in addition to the NCLDV members, there are other viruses that qualify as giruses, including bacterial viruses such as Phage G and a virus called white spot syndrome virus, which causes a disease of major economic importance in cultured shrimp. With research efforts still in the early stages, giant viruses are already producing scientific and economics benefits. Novel enzymes are being discovered that have commercial value based on their functions and also based on the fact that viral enzymes are often the smallest in their class, making them ideal models for mechanistic and structural studies. At present, one obstacle to studying giruses is that none of them can be genetically modified by molecular techniques. Hopefully this barrier will fall soon.

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